Acetylcholine and Spontaneous Recognition Memory in Rodents and Primates

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Acetylcholine has long been linked to a role in memory (Drachman, 1977; Hasselmo, 2006a; Micheau & Marighetto, 2011), with loss of the transmitter evident in the early stages of Alzheimer's disease associated specifically with memory loss (Bierer et al., 1995). In particular, the projections of cholinergic cells from the basal forebrain to the cerebral cortex and hippocampus rising within the medial septum (MS) and vertical limb of the diagonal band (vDB) have been linked to memory in monkeys (e.g. Easton, Ridley, Baker, & Gaffan, 2002; Fine et al., 1997; Ridley, Barefoot, Maclean, Pugh, & Baker, 1999) and lesions of the basal forebrain produce a profound amnesia in humans (e.g. Deluca & Diamond, 1995; Norlen & Olivecrona, 1953). However, cholinergic cells are not the only cells present within this region of the basal forebrain, and damage in animals and humans has rarely been restricted to the MS/VDB and even more rarely to only the cholinergic projections from this region. As a result, the necessary involvement of these cholinergic cells in memory has been much debated (e.g. Baxter & Chiba, 1999; Easton, Douchamps, Eacott, & Lever, 2012; Hasselmo, 2006b; Parent & Baxter, 2004), with evidence that the role of acetylcholine may be more specific to attentional mechanisms than memory per se (e.g. Baxter & Chiba, 1999).

It is therefore very important when assessing the impact of cholinergic manipulations on memory to consider what is meant by memory and what is being modelled. As in much of neuroscience, the issue of translation from animal research into the clinic is a key concern. It is essential that we move away, therefore, from considering the debate to be one of acetylcholine's involvement in broadly described terms of 'memory' or 'attention'. Rather, we would argue that one needs to look carefully at the precise nature of the behavioural task being used (Ameen-Ali, Easton, & Eacott, 2015), and the nature of the behavioural impairment. Specific hypotheses about the nature of acetylcholine's function can be best arrived at through the careful consideration of specific elements of behaviour and its relation to the manipulation at hand (A. Easton, Douchamps, et al., 2012).

If we intend to model the clinical concerns of memory loss in ageing and Alzheimer's, then we need to consider specific aspects of memory. In particular, early stages of Alzheimer's (those which are associated primarily with a specific decline in cholinergic markers; Bierer et al., 1995) are associated with loss of episodic memory (Collie & Maruff, 2000). Episodic memory is the memory for specific, personally experienced events in one's life (Tulving, 1983). In humans it usually comes with the explicit conscious experience of recollecting and reliving the experience as it was originally experienced (so called 'mental time travel'; Suddendorf & Corballis, 2007). The element of conscious experience in episodic memory has led some to argue that it is a uniquely human form of memory (Suddendorf & Corballis, 2007; Tulving, 2002). However, many researchers have now presented behavioural models of episodic memory in a wide range of non-human species (e.g. Babb & Crystal, 2006; Clayton & Dickinson, 1998; M J Eacott & Norman, 2004; Madeline J Eacott, Easton, & Zinkivskay, 2005; Ferkin, Combs, delBarco-Trillo, Pierce, & Franklin, 2008; Kart-Teke, De Souza Silva, Huston, & Dere, 2006; Singer & Zentall, 2007) meaning that we can now explore the role of acetylcholine in this specific form of memory.

A content-based approach to episodic memory

In humans, episodic memory is primarily associated with the conscious experience of recollection. When one remembers what one ate for breakfast you remember it not in isolation, but as a relived experience, remembering who was there, what time it was, the taste and smells, the emotions of being rushed getting ready for work etc. In Tulving's original description of episodic memory he described it as memory which 'receives and stores information about temporally dated episodes or events, and tempero-spatial relations between them' (Tulving, 1983). The conscious reliving of this experience has been termed 'mental time travel', and it is this critical inclusion of conscious re-experience of the memory which pushes some towards the view that only humans are capable of episodic memories (Suddendorf & Corballis, 2007). Without being able to conclusively be persuaded that non-human animals have a conscious experience it is impossible to conclude that they have a form of memory so intrinsically tied to it.

However, conscious experience occurs for all sorts of cognitive phenomena, yet it does not impact on the description of the cognitive process to the same degree as it does in episodic memory. When we see an object we have a clear conscious experience of perception, and yet this has not prevented us from using animal models to carefully explore the neural basis of such perception, even when the conscious experience of the animal model cannot be understood. In short, one would not deny that a monkey or a rat can see, just because they may not have the conscious experience afforded to humans when they see. Therefore the question remains why animals are not easily afforded the concept of episodic memory purely on the basis of the potential absence of a conscious experience.

As a result of this limitation imposed by consciousness, Clayton and Dickinson (1998) proposed an alternative approach to defining episodic memory in a way that could be modeled outside of humans. Their demonstration of what-where-when (WWW) memory in scrub jays showed that these birds could adapt their behavioural response to a particular food (what; worms or peanuts) they had cached in a particular location (where), and at a particular time (recently or several days previously). They argued that this memory of what happened, where and when met Tulving's description of episodic memory. Also, there are still additional criteria which need to be met to ensure such a content-based description truly captures the essence of this clinically important form of memory. For example one might remember what (you were born), where and when, and yet this would not be an episodic memory as it is not the recollection of a personally experienced event, rather it stems from semantic memory. Therefore Clayton et al (Clayton, Bussey, & Dickinson, 2003) set out a series of other criteria that were required for episodic memory to be demonstrated in animals, including its structure (which must be an integrated single memory, not the combination of multiple memories) and flexibility to remember things with no explicit reason for knowing that they needed to be remembered.

This content-based description of episodic memory has become more widely accepted in recent years. However, what-where-when memory has not been consistently demonstrated across species, including non-human primates (Hampton, Hampstead, & Murray, 2005). One critical limit has been the importance of time to WWW memory. Although Tulving spoke of 'temporally dated' events, actual dating of memories in humans is very difficult and often relies on non-episodic information

(Friedman, 1993, 2007). Alternate versions of WWW were subsequently developed with the aim of maintaining the content of the memory (what happened, where and the occasion it happened on), but allowing the occasion to be defined in ways other than purely information about when it happened. In particular, context has been used to define one event as being separate from another (Eacott & Norman, 2004; Robertson, Eacott, & Easton, 2015).

In the what-where-which occasion task, Eacott and Norman (2004) used a spontaneous recognition task in rodents, where animals demonstrate their memory through preferential exploration of novel items. In exploring a novel item (or combination of features) they demonstrate their memory for having seen the more familiar item before (A Ennaceur & Delacour, 1988). In the first sample event, rats were exposed to two objects (e.g. A and B) in left and right positions within an open field with a particular visuo-tactile context present in the arena (e.g. context X; a metal mesh on the walls and floors of the arena). After a short delay a second sample event was presented in the same arena but with a new context present (e.g. context Y; a patterned and ridged plastic floor). The same objects (A and B) were presented again, but now in reversed positions (i.e. in context X, object A would be on the right of the arena, but in context Y it would be on the left of the arena). After a delay period the animal would be returned to the arena with one of the previous contexts present and two new copies of one of the previously seen objects. For example, the rat may have been returned to context X and seen two new copies of object A in the left and right positions. In this case the context is familiar, as is the object, the combination of object A on the left and on the right, and in context X. However, there is novelty in this test stage as in this particular example when object A was seen in context X it was on the right hand side of the arena. Therefore the presence of object A on the left side of the arena in context X is novel (A has only been seen on the left in context Y previously). Therefore novelty in this task is not defined by an individual feature (object, location or context), but rather as a coherent single memory of what has been seen, where it has been seen and which occasion (context X or Y) it was seen there.

The role of acetylcholine in what-where-which occasion memory

As discussed earlier, the importance of an animal model of episodic memory is that it provides a close match to the clinically relevant form of memory that is impaired early in diseases such as Alzheimer's (Collie & Maruff, 2000). In the case of the proposed relationship between acetylcholine and memory loss in Alzheimer's, the ability to explore this relationship in the type of memory loss seen in the clinic improves the ability to translate findings from animal to human studies.

Using an immunotoxic lesion (IgG-Saporin) designed to specifically target cells that express acetylcholine as a transmitter (Wiley, Oeltmann, & Lappi, 1991), Easton and colleagues investigated the role of specific cholinergic input to the hippocampus (Easton, Fitchett, Eacott, & Baxter, 2010). Within the basal forebrain where cholinergic projections arise, the MS/vDB project directly to the hippocampus (Mesulam, Mufson, Wainer, & Levey, 1983). Targeting these structures with the immunologic lesion therefore aimed to reduce cholinergic input specifically to the hippocampus. The hippocampus itself is known to be critical for both episodic memory in humans (e.g. Aggleton & Brown, 1999; Bayley & Squire, 2003; Scoville &

Milner, 1957) and for what-where-which occasion memory in rodents (Eacott & Norman, 2004; Langston & Wood, 2010). However, cholinergic depletion of the hippocampus had no effect on this episodic memory task (Easton et al., 2010). The lesion was selective for acetylcholine (GABAergic cells were reliably intact following the lesion), and effective as another behavioural task was found to be impaired in these same animals (a where-which task, see below). The lack of impairment in the episodic task was also not simply a result of the lesion being slow to develop as returning to the episodic task after seeing an impairment in the where-which task still showed no impairment in what-where-which occasion memory (Easton et al., 2010).

The where-which task impaired in these animals was another spontaneous recognition memory task, but this time novelty was defined by the combination of location and context (i.e. at test one location was filled with an object which had not been previously occupied in that context at sample - but had been occupied in a sample with another context). The task is based on spatial-context conditional discriminations in reward-based tasks which had previously been shown to be impaired following cholinergic lesions to the hippocampus in both marmosets (Ridley et al., 1999) and rats (Janisiewicz, Jackson, Firoz, & Baxter, 2004).

As a result, the pattern of results from this study leave us with two unusual observations. First, the hippocampus is necessary for both the episodic memory task and the where-which task, and yet cholinergic inputs to the hippocampus are necessary only for the where-which task. This means that there must be dissociation of function within the hippocampus on the basis of cholinergic input. Some hippocampal tasks rely on acetylcholine and some don't and these tasks can be manipulated independently of each other. However, with only a single dissociation in evidence it remains possible that some simpler explanation remains for this pattern of results, such as task difficulty, with only more difficult tasks being sensitive to the removal of acetylcholine. However, in the case of this current set of data this would require the two component where-which task to be *more* difficult than the three component what-where-which occasion task, even though there are overlapping features between the tasks. Indeed, the discrimination ratio in both tasks is very similar in these animals (Easton, Fitchett, Baxter, & Eacott, 2009) implying that the episodic task is not obviously more difficult as control animals are equally able to show memory ability in both tasks. Such a task difficulty explanation therefore remains unlikely, leaving us to conclude that there is dissociation within the hippocampus based on the necessity for acetylcholine in performing memory tasks. The hippocampus is not the only site where such dissociation on the basis of cholinergic involvement is seen. Only a portion of tasks dependent on the pre-frontal cortex in macaques depend on the cholinergic projections to pre-frontal regions (Croxson, Kyriazis, & Baxter, 2011).

The second unusual observation from this data is that procedures similar to the what-where-which occasion task have been impaired following cholinergic lesions in non-human primates (Easton et al., 2002). In a scene learning task, monkeys were taught a visual discrimination task (to simply learn which one of two objects presented were correct by trial and error), and these discriminations took place against a background scene. Each time the visual discrimination problem was presented the same objects were in the same locations against the same trial unique background. New problems were presented against a different background and with

the objects in different spatial locations. This scene task has also been argued to model episodic memory in monkeys (Gaffan, 1994), is reliant on the hippocampus (Gaffan, 1994) and requires the animals to solve problems with the content of object (what), location (where) and background scene (which occasion). It seems, then, that the results of immunotoxic lesions in primates impairing this episodic task (Easton et al., 2002) but the same lesions showing no effect on a similar episodic task in rats (Easton et al., 2010) causes some problems in interpretation.

These tasks of episodic memory in primates and rats are somewhat different, despite the apparent similarity in their content. In rodents, the what-where-which occasion task is one of spontaneous recognition. Animals require no training to perform the task and are not rewarded for their behavioural choices. In contrast, the scene learning task in monkeys is one of visual discrimination and therefore requires that animals learn to choose one object over another in order to achieve maximum food reward. In humans, episodic memory is spontaneous and requires no explicit effort to encode information. It is possible, then, that this difference in reward motivation and learning between the rodent and primate task is sufficient to explain the difference in outcome following cholinergic lesions. However, both the primate scene learning task and the spontaneous rodent episodic memory task have been adapted for use in humans, and they show either phenomenological similarity to episodic memory (Easton, Webster, & Eacott, 2012) or impairment in amnesic patients (Aggleton et al., 2000). Subtle differences between the tasks then seem unlikely to cause such significant differences in the effect of cholinergic lesions.

A more likely cause of the difference between the results in rodents and primates is the scale of the cholinergic lesion used. In the rodent task the cholinergic lesion was targeted at the hippocampus, with the lesion made in the MS/vDB that projects directly to the hippocampus. However, in the primate studies, lesions extended beyond the MS/vDB and into the nucleus basalis of Meynert (nBM), meaning cortical regions including the perirhinal cortex were also depleted of their cholinergic input. Although the primate scene learning task is dependent upon the hippocampus (Gaffan, 1994), structures in the temporal and medial temporal cortices are also necessary (Easton & Gaffan, 2000; Murray, Baxter, & Gaffan, 1998). It is unclear, then, whether in primates cholinergic lesions of the hippocampus alone may have impaired the scene learning task, or whether a lack of impairment would have been seen, as in the rodents' episodic memory task. Similarly, it remains unclear whether a more widespread lesion of the cholinergic system in rodents might have produced an impairment in the episodic memory task.

Differential roles of acetylcholine in the hippocampus and perirhinal cortex

Whether cholinergic input to both the hippocampus and perirhinal cortex is necessary for episodic memory in animals is an important question, as cholinergic inputs to these two regions are known to have very different patterns of impairment. In rats, i.p. injection of scopolamine (which will have central effects across both regions) impairs spontaneous recognition memory, although only at higher doses than those which impaired the same animals on a radial maze spatial learning task (Ennaceur & Meliani, 1992). Barros and colleagues have recently shown object location memory deficit in marmosets as well after scopolamine was given ip, but at doses that also impaired their contextual fear-conditioning (Melamed, de Jesus,

Maior, & Barros, 2017). Closer investigation shows that whilst ip scopolamine impairs spontaneous recognition, it only does so when delivered during the encoding (sample) phase, but not when administered during the retrieval (test) phase (Melamed et al., 2017; Warburton et al., 2003). Scopolamine-induced impairment in rat spontaneous object recognition and marmoset object discrimination can also be reversed by a number of nootropic drugs (e.g. rat: Milić et al., 2013; Rutten, Prickaerts, & Blokland, 2006; Woolley et al., 2009; marmoset: Carey et al., 1992).

Direct infusion of scopolamine into the perirhinal cortex mirrors the effects of systemic administration in impairing object recognition (rat: Warburton et al., 2003; macaque: Tang, Mishkin, & Aigner, 1997), and so the impairments from systemic administration cannot be ascribed simply to peripheral effects which might serve to cause particular confounds in a task of spontaneous exploratory behaviour. In contrast, scopolamine infusions into the hippocampus produce impairments in spatial memory (e.g. Blokland, Honig, & Raaijmakers, 1992; Givens & Olton, 1995)

Immunotoxic lesions of the cholinergic projections to either the perirhinal cortex or hippocampus mirror the effects of direct scopolamine administration. Cholinergic lesions of the perirhinal cortex through direct injections into the cortex impair object recognition memory in rats (Winters & Bussey, 2005). Perirhinal lesions in macaques (Turchi, Saunders, & Mishkin, 2005) and nBM lesions in marmosets (Ridley et al., 1999) lead to similar object discrimination impairments. In contrast, specific cholinergic lesions of the hippocampus produce a reliable impairment in spontaneous recognition of spatial locations in rats (Cai, Gibbs, & Johnson, 2012) and visuospatial discriminations in marmosets (Ridley et al., 1999). Cholinergic agents can minimize these immunotoxin-induced performance impairments (Ridley et al., 1999).

Does the what-where-which task measure episodic memory?

Given the impact of cholinergic manipulations on spontaneous recognition of objects, locations and object-locations, the lack of impairment in what-where-which stands out. The perirhinal cortex supports object recognition (Eacott & Gaffan, 2005; Murray et al., 1998) and cholinergic inputs to perirhinal cortex are required for this, whether in spontaneous recognition tasks in rodents (Winters & Bussey, 2005) or in rewarded object recognition tasks such as delayed match to sample in primates (Turchi et al., 2005). Similarly the hippocampus is required for many spatial learning tasks and at least some of these tasks are also dependent upon the cholinergic inputs to the hippocampus (e.g. Cai et al., 2012). However, the episodic memory task (what-where-which) appears to be different in that it requires the hippocampus (Eacott & Norman, 2004; Langston & Wood, 2010) but not the cholinergic inputs to the hippocampus (Easton et al., 2010).

In humans there is data that implicates the cholinergic system in episodic memory particularly. Lesions within the basal forebrain give rise to significant amnesia (e.g. Deluca & Diamond, 1995), although cholinergic cells will not be the only types affected by such lesions. However, in the early stages of Alzheimer's disease, where episodic memory is primarily affected (Collie & Maruff, 2000), it is biomarkers of cholinergic activity that best predict memory performance (Bierer et al., 1995). One possibility, then, is that the what-where-which task in rodents simply does not

measure episodic memory and that the content-based approach to modelling episodic memory may not be sufficient. However, there are several reasons to believe this is not the case.

As discussed above, any task of episodic memory should meet a number of criteria, not just show what-where-which occasion content (Clayton et al., 2003). One of those criteria is that the memory should be a single coherent memory, and not the result of summation of independent memories for different components of the event (such as what or where) on their own. The very lack of impairment in the what-where-which task strongly suggests that the memory is not the result of simple summations of component memories. Although manipulations of the cholinergic system in the hippocampus do not impair the episodic memory task, they do impair where-which memory (Easton et al., 2010). If the episodic memory task were merely a summation of smaller component tasks then the failure to be able to process some of these components (e.g. where-which memory) should prevent the overall completion of a task requiring those components. That where-which recognition and what-where-which recognition are dissociable in terms of their requirement for acetylcholine in the hippocampus shows us that the episodic memory task uses a single coherent memory for the entire event rather than just combining components together.

Further evidence that the what-where-which task measures episodic memory comes from human data. Human participants run on versions of the rodent what-where-which task have shown that these memories require recollection and cannot be solved using familiarity alone, even though a what-where-when version of the same task can be, implying a clear link to episodic memory processes (Easton, Webster, et al., 2012; Persson, Ainge, & O'Connor, 2016). In addition, we have run human participants on an object recognition task in which participants only have to make old or new judgements about individual objects. However, without it being necessary for solving the object recognition task aspects of the location, the background context or both were altered between encoding and retrieval of the object memory. In this case the degree to which recollection was used (compared to familiarity) increased markedly when the spatial location of the object and the background context were identical at encoding and retrieval, whilst matching either location or background context on its own did not lead to the same increase in recollection (Ameen-Ali, Norman, Eacott, & Easton, 2017). Together these studies show that in humans, what-where-which memories rely on recollection and phenomenologically appear very similar to episodic memory as defined through non-content based descriptions.

Together, then, it appears very unlikely that the lack of impairment in the what-where-which task in rodents with lesions of the cholinergic input to the hippocampus is a result simply of a mismatch between the task and the cognitive process it aims to model. Rather, we may be able to explain the lack of impairment in the episodic memory task by looking to the role of acetylcholine in encoding and retrieval.

The role of acetylcholine in encoding and retrieval

Acetylcholine is released during exposure to novelty, and higher levels of acetylcholine boost a wide array of novelty-oriented processes, such as exploration (e.g rearing on hind legs), and synaptic plasticity, reviewed in (Easton, Douchamps,

et al., 2012; Hasselmo, 2012; Lever, Burton, & O'Keefe, 2006; Poulter, Hartley, & Lever, 2018). One of acetylcholine's effects is to reduce proactive interference, i.e. interference from previously encoded associations. Scopolamine administration to the perirhinal cortex impairs object recognition memory when given at encoding, but not at retrieval (Warburton et al., 2003). This impairment of encoding but not retrieval is also a common outcome of scopolamine administration in other domains such as hippocampal-dependent spatial memory (Deiana, Platt, & Riedel, 2011; Easton, Douchamps, et al., 2012), and sits alongside observations of the role of acetylcholine in interference (Winters, Bartko, Saksida, & Bussey, 2007). When interfering stimuli are presented (ie stimuli similar to those used in the experiment irrelevant to the experiment) in the presence of scopolamine administration there is a surprising improvement in object recognition memory (Winters, Saksida & Bussey, 2006). This effect has been attributed to acetylcholine's involvement in encoding all object information. If information about irrelevant objects is encoded after the experimental encoding stage then this can interfere with the experimentally relevant memories. In contrast, if scopolamine is administered when these interfering stimuli are presented then they will fail to be encoded well, and therefore will have a lesser interfering effect on the experimental stimuli meaning those experimental stimuli will be better remembered as a result.

These interference-related problems have been seen in computational modelling of encoding and retrieval and have led to a set of high profile models of the way in which acetylcholine allows the separation of encoding and retrieval states (Douchamps, Jeewajee, Blundell, Burgess, & Lever, 2013; Hasselmo, 1999, 2006a, 2012; Meeter, Murre, & Talamini, 2004). By suppressing recurrent inputs within the hippocampus, notably those mediated by region CA3, interference can be reduced by preventing the retrieval of previously learned associations from pattern completion. Instead, pattern separation is encouraged allowing distinct items to be encoded separately from one another with reduced interference (Duncan, Sadanand, & Davachi, 2012; Hasselmo, 1999, 2006a; Meeter et al., 2004). In contrast, low levels of acetylcholine would then improve retrieval and consolidation of information, and such low levels can be seen in states such as slow wave sleep in which memory consolidation is thought to occur (Gais & Born, 2004).

Such models explain that in any task in which proactive interference is likely to occur, acetylcholine is important in order to help encode novel information in spite of interfering information. How might these models explain the role of acetylcholine in the hippocampus in a where-which but not a what-where-which task in rats (Easton, Fitchett, Eacott, & Baxter, 2011) where levels of interference might be expected to be very similar? Indeed, interference could be even higher in the *what-where-which* task as the same objects are experienced by the animal at each phase of the trial. However, in these animals only cholinergic inputs to the hippocampus are lesioned, and we know the hippocampus has a high level of involvement in spatial memory. In the *what-where-which* task, the location of a particular object changes across the trial and across contexts, but every time the animal goes into the arena objects are always to the left and right of the animal. As a result the purely spatial component of this memory does not change. In contrast, locations of objects in the *where-which* task constantly shift within the trial. On no two entries into the arena are objects in the same two locations. As a result there is more potential for *spatial* interference in this task as a series of entries into the arena have to be separated in memory by

distinguishing between highly similar but not identical locations within that arena. If acetylcholine in the hippocampus was particularly important for reducing the impact of potential interference in spatial memory then we might expect it to be of more importance in the where-which task than the what-where-which task because of the instability of spatial locations over trials in the where-which task (Easton, Douchamps, et al., 2012).

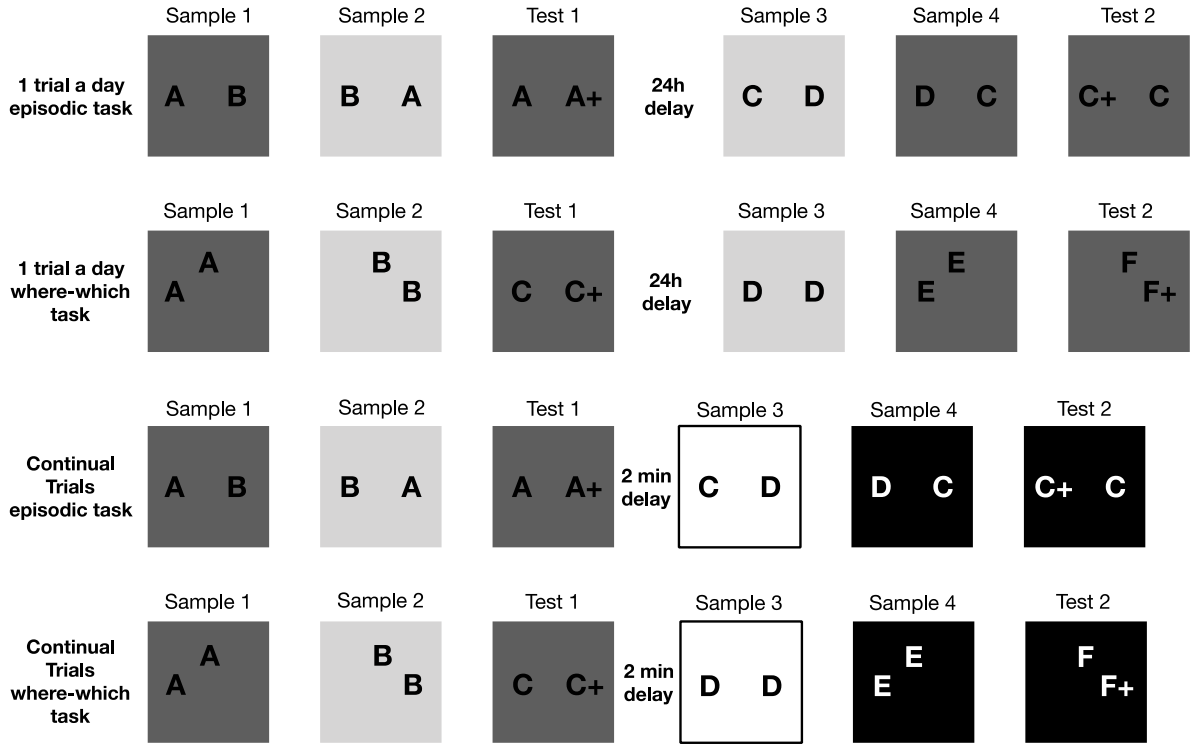

Figure 1: Schematic representations of the episodic (what-where-which) and where-which tasks in rats. For comparison 1 trial a day versions (top two panels) are presented where there is a 24h delay between trials and 16 trials are run over 16 days of testing. The continual trials version (bottom two panels) have only a 2 min delay between trials with all trials (16 in total) happening in a single session. To allow animals to distinguish the separate trials run within a continual trials session, more contexts are used in the continual trials versions of the task than the 1 trial a day versions. Objects are real world junk objects, with unique objects being identified by different letters in the figure above. In all cases + indicates the novel feature at test for the trial shown. Differences in spatial interference can be seen between the tasks with the location of objects fixed in left/right positions every time the animal enters the apparatus for the episodic memory task. In contrast the positions of objects vary not just on a trial by trial basis but also across samples and tests for the where-which task, leading to an increased need to maintain accurate representations of object locations in this task (Easton et al., 2010; Seel, Eacott, Langston, & Easton, 2018a).

To explicitly test this hypothesis, we recently investigated both the where-which and what-where-which tasks in rats with lesions of the cholinergic projections to the hippocampus, but in versions of those tasks where many trials were run consecutively rather than in a one trial a day manner (Seel, Eacott, Langston, & Easton, 2018b). By running the tasks using this continual trials approach (Ameen-Ali, Eacott, & Easton, 2012; Chan et al., 2018) we were able to raise the levels of proactive interference in both tasks. The nature of running many trials consecutively means that each trial is highly similar (with overlap of object features, contexts and spatial locations) and therefore proactive interference is a feature of the design, and can be seen in performance of normal animals on some tasks (Chan et al., 2018). However, although overall interference levels will have gone up in both task versions, because there are more trials than in standard versions of the tasks, it remains the case that spatial variability remains high only in the where-which task. As a result, if acetylcholine in the hippocampus was required to resolve all interference then we would expect the loss of acetylcholine in the hippocampus to impact on both tasks using continual trials. If on the other hand acetylcholine in the hippocampus is only necessary to resolve spatial interference then we would expect still to see a role for hippocampal acetylcholine in where-which memory but not in what-where-which memory, as before. We found that IgG-Saporin lesions of the MS/vDB in rats continued to only impair where-which memory and not what-where-which memory, even when run with this high level of interference (Seel et al., 2018b).

These findings support the idea that whilst the hippocampus is necessary for what-where-which memory, acetylcholine in the hippocampus is only required for identification of spatial novelty. This may also, then, explain the difference between the what-where-which task in rodents and the scene learning task in primates. The what-where-which task involves objects being presented in stable spatial locations within and across trials, meaning there is limited opportunity for interference in the spatial component of this memory (Easton, Douchamps et al., 2012; Easton et al., 2011). In contrast, the scene learning task in monkeys more closely resembles the where-which task in rats in that the locations of objects are trial unique and therefore these highly similar spatial locations need to be separated in memory. This separation requires the cholinergic system to promote encoding of separate locations despite high levels of spatial interference. With the use of spontaneous recognition tasks to explore cholinergic function across rodents (A Easton et al., 2011; Seel et al., 2018b; Winters & Bussey, 2005) and primates (Melamed et al., 2017) we will be able to make more reliable comparisons across species. In addition, evidence that spontaneous recognition tasks of episodic memory can be translated to episodic memory in humans (Ameen-Ali et al., 2017; Easton, Webster, et al., 2012) taking us to a position where we are now able to improve translation from animal studies to the clinic.

REFERENCES

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia and the hippocampal-anterior thalamic axis. *Behavioral and Brain Sciences*, *22*, 425–444.

Aggleton, J. P., McMakin, D., Carpenter, K., Hornak, J., Kapur, N., Halpin, S., … Gaffan, D. (2000). Differential cognitive effects of colloid cysts in the third ventricle that spare or compromise the fornix. *Brain*, *123*, 800–815.

Ameen-Ali, K. E., Eacott, M. J., & Easton, A. (2012). A new behavioural apparatus to reduce animal numbers in multiple types of spontaneous object recognition paradigms in rats. *Journal of Neuroscience Methods*, *211*(1). https://doi.org/10.1016/j.jneumeth.2012.08.006

Ameen-Ali, K. E., Easton, A., & Eacott, M. J. (2015). Moving beyond standard procedures to assess spontaneous recognition memory. *Neuroscience and Biobehavioral Reviews*, *53*. https://doi.org/10.1016/j.neubiorev.2015.03.013

Ameen-Ali, K. E., Norman, L. J., Eacott, M. J., & Easton, A. (2017). Incidental context information increases recollection. *Learning and Memory*, *24*(3). https://doi.org/10.1101/lm.042622.116

Babb, S. J., & Crystal, J. D. (2006). Episodic-like memory in the rat. *Current Biology*, *16*(13), 1317–1321.

Baxter, M. G., & Chiba, A. A. (1999). Cognitive functions of the basal forebrain. *Current Opinion in Neurobiology*, *9*, 178–183.

Bayley, P. J., & Squire, L. R. (2003). The medial temporal lobe and declarative memory. *International Congress Series*, *1250*, 245–259. Retrieved from http://www.sciencedirect.com/science/article/B7581-49N7DHR-R/2/0390d73876c3ab1dda8cf1533659f027

Bierer, L. M., Haroutunian, V., Gabriel, S., Knott, P. J., Carlin, L. S., Purohit, D. P., … Davis, K. L. (1995). Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits. *Journal of Neurochemistry*, *64*, 749–760.

Blokland, A., Honig, W., & Raaijmakers, W. G. M. (1992). Effects of intra-hippocampal scopolamine injections in a repeated spatial acquisition task in the rat. *Psychopharmacology*, *109*(3), 373–376. https://doi.org/10.1007/BF02245886

Cai, L., Gibbs, R. B., & Johnson, D. A. (2012). Recognition of novel objects and their location in rats with selective cholinergic lesion of the medial septum. *Neuroscience Letters*, *506*(2), 261–265. https://doi.org/10.1016/j.neulet.2011.11.019

Carey, G. J., Costall, B., Domeney, A. M., Gerrard, P. A., Jones, D. N. C., Naylor, R. J., & Tyers, M. B. (1992). Ondansetron and arecoline prevent scopolamine-induced cognitive deficits in the marmoset. *Pharmacology Biochemistry and Behavior*, *42*(1), 75–83. https://doi.org/10.1016/0091-3057(92)90449-P

Chan, M., Eacott, M. J., Sanderson, D. J., Wang, J., Sun, M., & Easton, A. (2018). Continual Trials Spontaneous Recognition Tasks in Mice: Reducing Animal Numbers and Improving Our Understanding of the Mechanisms Underlying Memory. *Frontiers in Behavioral Neuroscience*, *12*, 214. https://doi.org/10.3389/fnbeh.2018.00214

Clayton, N S, Bussey, T. J., & Dickinson, A. (2003). Can animals recall the past and plan for the future? *Nature Reviews Neuroscience*, *4*(8), 685–691. https://doi.org/10.1038/nrn1180

Clayton, N S, & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature*, *395*, 272–274.

Clayton, Nicola S., & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature*, *395*(6699), 272–274. https://doi.org/10.1038/26216

Collie, A., & Maruff, P. (2000). The neuropsychology of preclinical Alzheimer's disease and mild cognitive impairment. *Neuroscience and Biobehavioural Reviews*, *24*, 365–374.

Croxson, P. L., Kyriazis, D. A., & Baxter, M. G. (2011). Cholinergic modulation of a specific memory function of prefrontal cortex. *Nature Neuroscience*, *14*(12), 1510–1512. https://doi.org/10.1038/nn.2971

Deiana, S., Platt, B., & Riedel, G. (2011). The cholinergic system and spatial learning. *Behavioural Brain Research*, *221*(2), 389–411. https://doi.org/DOI 10.1016/j.bbr.2010.11.036

Deluca, J., & Diamond, B. J. (1995). Aneurysm of the anterior communicating artery - a review of neuroanatomical and neuropsychological sequelae. *Journal of Clinical and Experimental Neuropsychology*, *17*(1), 100–121. https://doi.org/10.1080/13803399508406586

Douchamps, V., Jeewajee, A., Blundell, P., Burgess, N., & Lever, C. (2013). Evidence for Encoding versus Retrieval Scheduling in the Hippocampus by Theta Phase and Acetylcholine. *Journal of Neuroscience*, *33*(20), 8689–8704. https://doi.org/10.1523/JNEUROSCI.4483-12.2013

Drachman, D. A. (1977). Memory and cognitive function in man - does cholinergic system have a specific role. *Neurology*, *27*(8), 783–790.

Duncan, K., Sadanand, A., & Davachi, L. (2012). Memory's penumbra: episodic memory decisions induce lingering mnemonic biases. *Science (New York, N.Y.)*, *337*(6093), 485–487. https://doi.org/10.1126/science.1221936

Eacott, M J, & Gaffan, E. A. (2005). The roles of perirhinal cortex, postrhinal cortex, and the fornix in memory for objects, contexts, and events in the rat. *Quarterly Journal of Experimental Psychology, B*, *58*, 202–217.

Eacott, M J, & Norman, G. (2004). Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? *The Journal of Neuroscience*, *24*(8), 1948–1953.

Eacott, Madeline J, Easton, A., & Zinkivskay, A. (2005). Recollection in an episodic-like memory task in the rat. *Learning & Memory*, *12*(3), 221–223. https://doi.org/10.1101/lm.92505

Easton, A., Douchamps, V., Eacott, M., & Lever, C. (2012). A specific role for septohippocampal acetylcholine in memory? *Neuropsychologia*, *50*(13). https://doi.org/10.1016/j.neuropsychologia.2012.07.022

Easton, A., & Gaffan, D. (2000). Comparison of perirhinal cortex ablation and crossed unilateral lesions of the medial forebrain bundle from the inferior temporal cortex in the rhesus monkey: Effects on learning and retrieval. *Behavioral Neuroscience*, *114*(6). https://doi.org/10.1037/0735-7044.114.6.1041

Easton, A., Webster, L. A. D., & Eacott, M. J. (2012). The episodic nature of episodic-like memories. *Learning and Memory*, *19*(4). https://doi.org/10.1101/lm.025676.112

Easton, A, Fitchett, A., Baxter, M. G., & Eacott, M. J. (2009). Cholinergic lesions of the medial septum impair where-which memory but not episodic memory in the rat. *European Brain and Behavior Society*. EBBS.

Easton, A, Fitchett, A., Eacott, M. J., & Baxter, M. G. (2011). Medial Septal cholinergic neurons are necessary for context-place memory but not episodic-like memory. *Hippocampus*, *21*, 1021–1027.

Easton, A, Ridley, R. M., Baker, H. F., & Gaffan, D. (2002). Unilateral lesions of the cholinergic basal forebrain and fornix in one hemisphere and inferior temporal cortex in the opposite hemisphere produce severe learning impairments in rhesus monkeys. *Cerebral Cortex*, *12*(7), 729–736. Retrieved from http://dx.doi.org/10.1093/cercor/12.7.729

Easton, Alexander, Fitchett, A. E., Eacott, M. J., & Baxter, M. G. (2010). Medial septal cholinergic neurons are necessary for context-place memory but not episodic-like memory. *Hippocampus*, *000*, 1–7. https://doi.org/10.1002/hipo.20814

Ennaceur, A, & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. . *Behavioural Brain Research*, *31*, 47–59.

Ennaceur, Abdelkader, & Meliani, K. (1992). Effects of physostigmine and scopolamine on rats' performances in object-recognition and radial-maze tests. *Psychopharmacology*, *109*(3), 321–330. https://doi.org/10.1007/BF02245880

Ferkin, M. H., Combs, A., delBarco-Trillo, J., Pierce, A. A., & Franklin, S. (2008). Meadow voles, Microtus pennsylvanicus, have the capacity to recall the "what", "where", and "when" of a single past event. *Animal Cognition*, *11*(1), 147–159. https://doi.org/10.1007/s10071-007-0101-8

Fine, A., Hoyle, C., MacLean, C. J., Levatte, T. L., Baker, H. F., & Ridley, R. M. (1997). Learning impairments following injection of a selective cholinergic immunotoxin, ME20.4 IgG-saporin, into the basal nucleus of Meynert in monkeys. *Neuroscience*, *81*(2), 331–343. https://doi.org/10.1016/s0306-4522(97)00208-x

Friedman, W. J. (1993). Memory for the time of past events. *Psychological Bulletin*, *113*, 44–66.

Friedman, W. J. (2007). The meaning of "time" in episodic memory and mental time travel. *Behavioral and Brain Sciences*, *30*, 323.

Gaffan, D. (1994). Scene-specific memory for objects: a model of episodic memory impairment in monkeys with fornix transection. *Journal of Cognitive Neuroscience*, *6*, 305–320.

Gais, S., & Born, J. (2004). Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(7), 2140–2144. https://doi.org/DOI 10.1073/pnas.0305404101

Givens, B., & Olton, D. S. (1995). Bidirectional Modulation of Scopolamine-Induced Working Memory Impairments by Muscarinic Activation of the Medial Septal Area. *Neurobiology of Learning and Memory*, *63*(3), 269–276. https://doi.org/10.1006/NLME.1995.1031

Hampton, R. R., Hampstead, B. M., & Murray, E. A. (2005). Rhesus monkeys (Macaca mulatta) demonstrate robust memory for what and where, but not when, in an open-field test of memory. *Learning and Motivation*, *36*, 245–259.

Hasselmo, M. E. (1999). Neuromodulation: acetylcholine and memory consolidation. *Trends in Cognitive Sciences*, *3*(9), 351–359. https://doi.org/citeulike-article-id:4132068

Hasselmo, M. E. (2006a). The role of acetylcholine in learning and memory. *Curr Opin Neurobiol*, *16*(6), 710–715. https://doi.org/S0959-4388(06)00122-X [pii]10.1016/j.conb.2006.09.002

Hasselmo, M. E. (2006b). The role of acetylcholine in learning and memory. *Current Opinion in Neurobiology*, *16*(6), 710–715. https://doi.org/10.1016/j.conb.2006.09.002

Hasselmo, M. E. (2012). *How we remember: Brain mechanisms of episodic memory*. Cambridge, MA: The M.I.T. Press.

Janisiewicz, A. M., Jackson, O., Firoz, E. F., & Baxter, M. G. (2004). Environment-spatial conditional learning in rats with selective lesions of medial septal cholinergic neurons. *Hippocampus*, *14*(2), 265–273. https://doi.org/10.1002/hipo.10175

Kart-Teke, E., De Souza Silva, M. A., Huston, J. P., & Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning and Memory*, *85*(2), 173–182.

Langston, R. F., & Wood, E. R. (2010). Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus*, *20*(10), 1139–1153. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19847786

Lever, C., Burton, S., & O'Keefe, J. (2006). Rearing on hind legs, environmental novelty, and the hippocampal formation. *Reviews in Neuroscience*, *17*(1–2), 111–133. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16703946

Meeter, M., Murre, J. M. J., & Talamini, L. M. (2004). Mode shifting between storage and recall based on novelty detection in oscillating hippocampal circuits. *Hippocampus*, *14*(6), 722–741. https://doi.org/10.1002/hipo.10214

Melamed, J. L., de Jesus, F. M., Maior, R. S., & Barros, M. (2017). Scopolamine Induces Deficits in Spontaneous Object-Location Recognition and Fear-Learning in Marmoset Monkeys. *Frontiers in Pharmacology*, *8*, 395. https://doi.org/10.3389/fphar.2017.00395

Mesulam, M. M., Mufson, E. J., Wainer, B. H., & Levey, A. I. (1983). Central cholinergic pathways in the rat - an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience*, *10*(4), 1185–1201. https://doi.org/10.1016/0306-4522(83)90108-2

Micheau, J., & Marighetto, A. (2011). Acetylcholine and memory: A long, complex and chaotic but still living relationship. *Behavioural Brain Research*, *221*(2), 424–429. https://doi.org/10.1016/j.bbr.2010.11.052

Milić, M., Timić, T., Joksimović, S., Biawat, P., Rallapalli, S., Divljaković, J., … Savić, M. M. (2013). PWZ-029, an inverse agonist selective for α5 GABAA receptors, improves object recognition, but not water-maze memory in normal and scopolamine-treated rats. *Behavioural Brain Research*, *241*, 206–213. https://doi.org/10.1016/J.BBR.2012.12.016

Murray, E. A., Baxter, M. G., & Gaffan, D. (1998). Monkeys with rhinal cortex damage or neurotoxic hippocampal lesions are impaired on spatial scene learning and object reversals. *Behavioral Neuroscience*, *112*(6), 1291–1303. https://doi.org/10.1037/0735-7044.112.6.1291

Norlen, G., & Olivecrona, H. (1953). The treatment of aneurysms of the circle of Willis. *Journal of Neurosurgery*, *10*(4), 404–415. https://doi.org/10.3171/jns.1953.10.4.0404

Parent, M. B., & Baxter, M. G. (2004). Septohippocampal acetylcholine: Involved in but not necessary for learning and memory? *Learning & Memory*, *11*(1), 9–20. https://doi.org/10.1101/lm.69104

Persson, B. M., Ainge, J. A., & O'Connor, A. R. (2016). Disambiguating past events: Accurate source memory for time and context depends on different retrieval processes. *Neurobiology of Learning and Memory*, *132*, 40–48. https://doi.org/10.1016/J.NLM.2016.05.002

Poulter, S., Hartley, T., & Lever, C. (2018). The Neurobiology of Mammalian Navigation. *Current Biology*, *28*(17), R1023–R1042. https://doi.org/10.1016/J.CUB.2018.05.050

Ridley, R. M., Barefoot, H. C., Maclean, C. J., Pugh, P., & Baker, H. F. (1999). Different effects on learning ability after injection of the cholinergic immunotoxin ME20.4IgG-saporin

into the diagonal band of Broca, basal nucleus of Meynert, or both in monkeys. *Behavioral Neuroscience*, *113*(2), 303–315.
Robertson, B.-A., Eacott, M. J., & Easton, A. (2015). Putting memory in context: Dissociating memories by distinguishing the nature of context. *Behavioural Brain Research*, *285*. https://doi.org/10.1016/j.bbr.2014.10.045
Rutten, K., Prickaerts, J., & Blokland, A. (2006). Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. *Neurobiology of Learning and Memory*, *85*(2), 132–138. https://doi.org/10.1016/j.nlm.2005.09.002
Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery and Psychiatry*, *20*, 11–21.
Seel, S. V., Eacott, M. J., Langston, R. F., & Easton, A. (2018a). Cholinergic input to the hippocampus is not required for a model of episodic memory in the rat, even with multiple consecutive events. *Behavioural Brain Research*, *354*, 48–54. https://doi.org/10.1016/J.BBR.2017.06.001
Seel, S. V., Eacott, M. J., Langston, R. F., & Easton, A. (2018b). Cholinergic input to the hippocampus is not required for a model of episodic memory in the rat, even with multiple consecutive events. *Behavioural Brain Research*, *354*, 48–54. https://doi.org/10.1016/J.BBR.2017.06.001
Singer, R. A., & Zentall, T. R. (2007). Pigeons learn to answer the question “where did you just peck?” and can report peck location when unexpectedly asked. *Learning & Behavior*, *35*(3), 184–189.
Suddendorf, T., & Corballis, M. C. (2007). The evolution of foresight: What is mental time travel, and is it unique to humans? *Behavioral and Brain Sciences*, *30*(3), 299-+. https://doi.org/10.1017/s0140525x07001975
Tang, Y., Mishkin, M., & Aigner, T. G. (1997). Effects of muscarinic blockade in perirhinal cortex during visual recognition. *Proceedings of the National Academy of Sciences*, *94*(23), 12667–12669. https://doi.org/10.1073/PNAS.94.23.12667
Tulving, E. (1983). *Elements of episodic memory*. London: Oxford University Press.
Tulving, E. (2002). Episodic memory: from mind to brain. *Annual Review of Psychology*, *53*, 1–25.
Turchi, J., Saunders, R. C., & Mishkin, M. (2005). Effects of cholinergic deafferentation of the rhinal cortex on visual recognition memory in monkeys. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(6), 2158–2161. https://doi.org/10.1073/pnas.0409708102
Warburton, E. C., Koder, T., Cho, K., Massey, P. V, Duguid, G., Barker, G. R. I., … Brown, M. W. (2003). Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. *Neuron*, *38*(6), 987–996. https://doi.org/10.1016/S0896-6273(03)00358-1
Wiley, R. G., Oeltmann, T. N., & Lappi, D. A. (1991). Immunolesioning - selective destruction of neurons using immunotoxin to rat NGF receptor . *Brain Research*, *562*(1), 149–153. https://doi.org/10.1016/0006-8993(91)91199-b
Winters, Boyer D., Saksida, L.M., Bussey, T. J. (2006). Paradoxical Facilitation of Object Recognition Memory after Infusion of Scopolamine into Perirhinal Cortex: Implications for Cholinergic System Function. *Journal of Neuroscience*, *26*, 9520–9529.
Winters, B. D., Bartko, S. J., Saksida, L. M., & Bussey, T. J. (2007). Scopolamine infused into perirhinal cortex improves object recognition memory by blocking the acquisition of

interfering object information. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *14*(9), 590–596. https://doi.org/10.1101/lm.634607

Winters, B. D., & Bussey, T. J. (2005). Removal of cholinergic input to perirhinal cortex disrupts object recognition but not spatial working memory in the rat. *European Journal of Neuroscience*, *21*(8), 2263–2270. https://doi.org/10.1111/j.1460-9568.2005.04055.x

Woolley, M. L., Waters, K. A., Gartlon, J. E., Lacroix, L. P., Jennings, C., Shaughnessy, F., … Dawson, L. A. (2009). Evaluation of the pro-cognitive effects of the AMPA receptor positive modulator, 5-(1-piperidinylcarbonyl)-2,1,3-benzoxadiazole (CX691), in the rat. *Psychopharmacology*, *202*(1–3), 343–354. https://doi.org/10.1007/s00213-008-1325-2